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STUDIES ON MOLECULAR CHARACTERISATION OF ELITE BC_3F_3 PROGENIES FOR SORGHUM DOWNY MILDEW RESISTANCE IN MAIZE (*ZEA MAYS* L.)

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ABSTRACT

Downy mildews, caused by several species in the genera Peronosclerospora and Sclerophthora, represent a destructive systemic disease of major economic importance to maize. Sorghum downy mildew caused by Peronosclerospora sorghi is considered as the most important biotic constraint limiting maize productivity. Cost concerns and the emerging problem of chemical resistance buildup in the pathogen point to the use of resistant varieties as a cost-effective and environmentally safe alternative for managing the disease. Two maize inbred lines UMI 79and UMI 936(w) with varied responses to sorghum downy mildew incidence were crossed and the resultant progenies were backcrossed with the recurrent parent, UMI 79 for introgression of sorghum downy mildew resistant genes into the elite inbred. Three BC_3F_1 progenies (7-2-3, 7-7-7 and 7-2-10) carrying the SDM resistant QTL selected based on the heterozygosity produced by the polymorphic SSR markers were selectively selfed to generate the BC_3F_2 population. The three progenies were phenotypically screened for sorghum downy mildew resistance in sick plot condition. Progeny 7-2-10 was found to be highly susceptible to the disease and thus eliminated from further studies. Progeny 7-7-7 and 7-2-3 showed moderate level of resistance with progeny 7-7-7 showing higher resistance to sorghum downy mildew.

In the present study thirty eight phenotypically resistant individuals from 7-2-3 progeny and sixty five phenotypically resistant individuals from progeny no. 7-7-7 were selfed to generate the BC_3F_3 generation. Among these eight superior progenies were selected for genotyping with the SSR markers phi053 (chromosome 3) and nc013 (chromosome 6). One BC_3F_3progeny (7-7-7-53) was identified homozygous for UMI 936(w) allele for both the markers and two progenies (7-7-7-85 and 7-2-3-8) were identified homozygous for UMI 936(w) allele for marker nc013 alone. These three lines can be designated as NILs (Near Isogenic Lines) resistant to the sorghum downy mildew disease and can be used in further breeding programmes. Four BC_3F_3 progenies (7-7-7-59, 7-7-7-84, 7-7-7-86 and 7-2-3-2) were identified heterozygous for the markers. They are to be selfed and advanced into next generation to identify more NILs differing for the resistance QTL alone.

KEYWORDS: Maize, Sorghum Downy Mildew, Backcross, Genotyping, Molecular Characterization, Resistant

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Original Article

INTRODUCTION

Maize (*Zea mays* L.) is one of the most versatile crops having wide adaptability under varied agro-climatic conditions. In India, maize is the third most important food crops after rice and wheat. It is cultivated in an area of 75.90 lakh hectares out of which Tamil Nadu contributes around 12.20 lakh hectares. To meet the increasing demand for maize, we need to increase and stabilize the maize production while maintaining the quality of the produce. Biotic stresses are one of the most limiting factors for stable crop production worldwide.

Minimizing losses from biotic stresses offer tremendous opportunities for increasing and stabilizing maize productivity along with maintaining the quality of the seeds/grains.

Downy mildew is considered the highest priority as a biotic constraint limiting maize productivity (Pingali, and Pandey, 2001). The important species causing downy mildew in maize in India are the sorghum downy mildew (SDM; *Peronosclerospora sorghi*), brown stripe downy mildew (BSDM; *Sclerophthora rayssiae* var. *zeae*) and Rajasthan downy mildew (RDM; *Peronosclerospora hetropogoni*).

Sorghum downy mildew (SDM) is particularly prevalent in the peninsular of India, in the states of Karnataka, Tamilnadu and Andhra Pradesh (Krishnappa *et al*., 1995). The fungus can spread locally through movement of spores in the air. The disease can be seed-borne and longer distance dispersal may be possible through movement of infected seed or spore-contaminated soil. The pathogen infects the roots primarily by oospores and the leaves by conidia and finally reaches the meristem causing systemic infection.

This disease can occur at any stage of maize development from seedling to harvest, though it primarily infects its host soon after seedling emergence, until one month after planting. The disease is known by two names, “downy mildew” and “crazy top” based on two types of symptoms in maize that develop as a result of systemic infection.

Though the disease can be controlled by cultural practices their effectiveness on disease incidence is variable and in most cases, offer incomplete control. Also the cost concerns and the emerging problem of a buildup of chemical resistance in the pathogen while using chemical control point to the use of resistant varieties as a more cost-effective and environmentally safe alternative for managing the problem of downy mildew in maize (Rathore and Jain, 2000).

The following objective was designed for the present investigation of backcross population from UMI 79 X UMI 936(w) cross:

- Sorghum downy mildew molecular characterisation in advanced backcross population of UMI 79 x UMI 936(w).
- Identification of backcross progenies having major QTLs responsible for downy mildew resistance using SSR markers.

MATERIAL AND METHODS

From BC_3F_1 population developed by repeated backcrossing involving UMI 79 (susceptible recurrent parent) with single plants carrying resistant QTLs identified through phenotyping and genotyping, three progenies *viz.*, 7-2-3, 7-7-7 and 7-2-10, heterozygous for the SSR markers were selectively selfed to generate the BC_3F_2 population. The BC_3F_2 population was raised under sorghum downy mildew sick plot conditions and phenotypically screened for the disease. Resistant progenies 7-2-3 and 7-7-7 were selfed to generate BC_3F_3 generation. The procedure adopted to develop sorghum downy mildew resistant lines is depicted in Fig. 1.

The present study includes identification of backcross progenies having major QTLs responsible for downy mildew resistance using SSR markers.

Source of Primers

The SSR markers used for chromosome 3 is phi053 and for chromosome 6 is nc013. Genetic limkage map showing SDM QTL location is given in Fig. 2 & 3. The information of SSR primers which is linked to sorghum downy

mildew resistance used in the study was obtained from maize database and sequence details are given in the Table 1.

SEASON	BACKCROSS BREEDING PROGRAMME	METHOD OF SCREENIG
Kharif' 09	UMI 79 (Susceptible) X UMI 936(w) (Resistant)	• Parental polymorphism survey using SSR markers
Kharif' 11	F1 X UMI 79 (Recurrent parent)	• Marker assisted foreground selection in F1 • One single true to type F1 hybrid selected and backcrossed
Rabi' 12	BC1F1 X UMI 79	• Phenotypic screening for sorghum downy mildew resistance • Marker assisted foreground selection • 14 BC_1F_1s selected and backcrossed
Kharif' 12	BC2F1 X UMI 79	• Phenotypic screening for sorghum downy mildew resistance • Marker assisted foreground selection • 5 BC_2F_1s selected and backcrossed
Rabi' 13	BC3F1	• Phenotypic screening for sorghum downy mildew resistance • Marker assisted foreground selection • 3 BC_3F_1s selected and selfed
Kharif' 13	BC3F2	• Phenotypic screening for sorghum downy mildew resistance • 104 resistant individual plants selfed
PRESENT STUDY		
Rabi'14	BC3F3	• 8 phenotypically superior progenies identified • Marker assisted foreground selection • Three NILs identified

Figure 1: The Procedure Adopted to Develop Sorghum Downy Mildew Resistant Lines

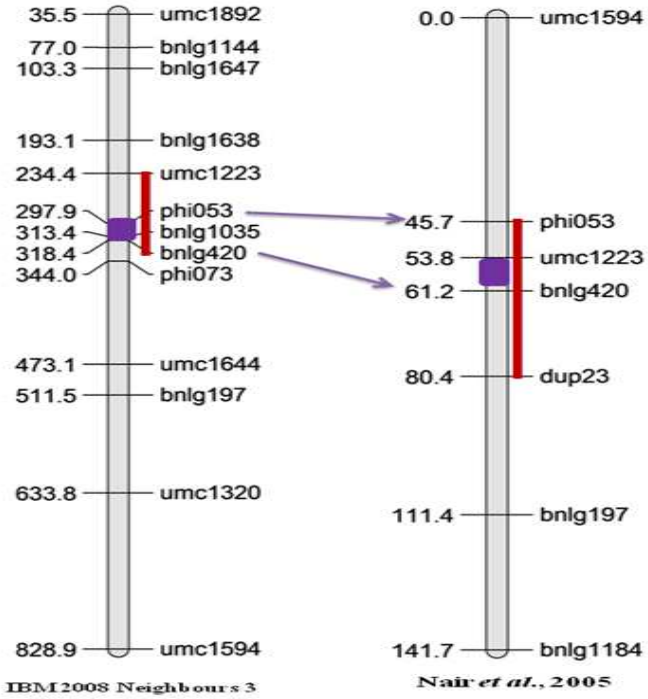

Figure 2: Genetic Linkage Map Showing Location of SDM QTL on Chromosome 3

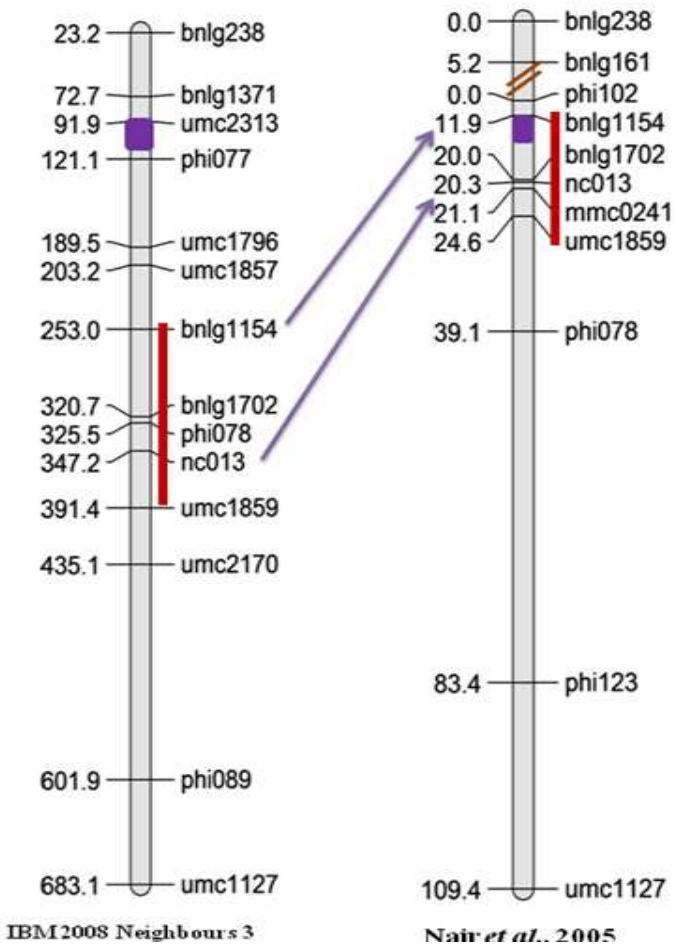

Figure 3: Genetic Linkage Map Showing Location of SDM QTL on Chromosome 6

Table 1: List of Foreground Markers Used for SSR Analysis

S. No.	SSR Marker	Chromosome Location	Sequence	Product Size (bp)
1	phi053	3	CTGCCTCTCAGATTCAGAGATTGAC (F) AACCCAACGTACTCCGGCAG (R)	180-196
2	nc013	6	AATGGTTTTGAGGATGCAGCGTGG (F) CCCCGTGATTCCCTTCAACTTTC (R)	114-134

Molecular Marker Analysis

The seedlings of BC_3F_3 were allowed to grow for 20 days in order to get enough leaf material for extraction of DNA. DNA was extracted from the selected genotypes following the procedure of CTAB method and quality check was done by Agarose gel electrophoresis. DNA was quantified by using Nanodrop. A total of two SSR primers one each from chromosome 3 (phi053) and chromosome 6 (nc013) were used for PCR amplification. Sixty eight BC_3F_3individuals obtained from eight cobs of the BC_3F_2 progenies were genotyped. After PCR amplification, the PCR products were separated by 3 %. The genotyping data of the BC_3F_3 lines were scored against the two SSRs. The segregation pattern of SSR markers for all the lines were scored as co-dominant fragments. The individuals showing the banding pattern similar to the parent, UMI 79 were scored as “A”, the heterozygous were scored as “H”, and the plants with the alleles similar to the parent, UMI 936(w) were scored as “B” and null bands were scored as “-”.

EXPERIMENTAL RESULTS

The SSR marker phi053 on chromosome 3 amplified the polymorphic segment of 180bp for UMI 79 and 196bp for UMI 936(w). The banding pattern obtained is given in Fig. 4. The marker nc013 on chromosome 6 amplified the polymorphic region between 114 bp for UMI 79 and 134bp for UMI 936(w). The identified heterozygotes were labelled as "H", individuals similar to female parent UMI 79 were labelled as "A" and individuals similar to the resistant donor parent UMI 936 were labelled as "B" (Figure 5).

Among the eight progenies studied, the progeny 7-7-7-53 alone had homozygous status for UMI 936(w) allele for both phi053 and nc013. Other progenies had heterogenous nature for either one or both alleles (Table 2).

From the BC_3F_3 population eight phenotypically superior progenies comprising a total of 68 individuals were selected for genotyping with the selected polymorphic markers of chromosome 3 and 6. The SSR markers *viz.*, phi053 located on the chromosome 3 and nc013 located on the chromosome 6 were found to show good polymorphism between the parental lines and used for genotyping among the selected BC_3F_3 progenies.

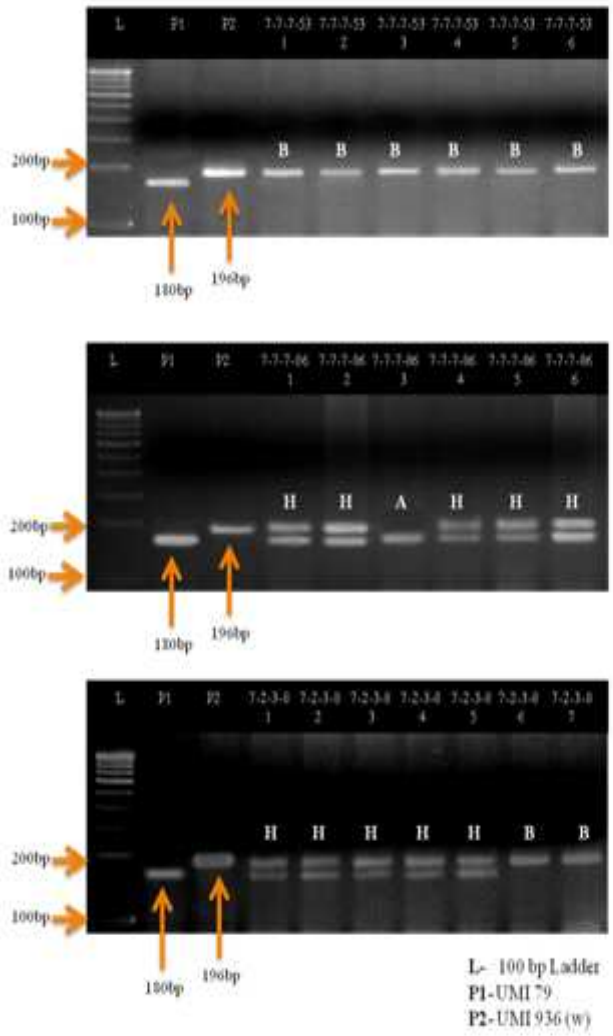

Figure 4: Segregation Pattern of SSR Matker phi053 in BC_3F_3 Progenies of (UMI79 x UMI936(w)) x UMI79

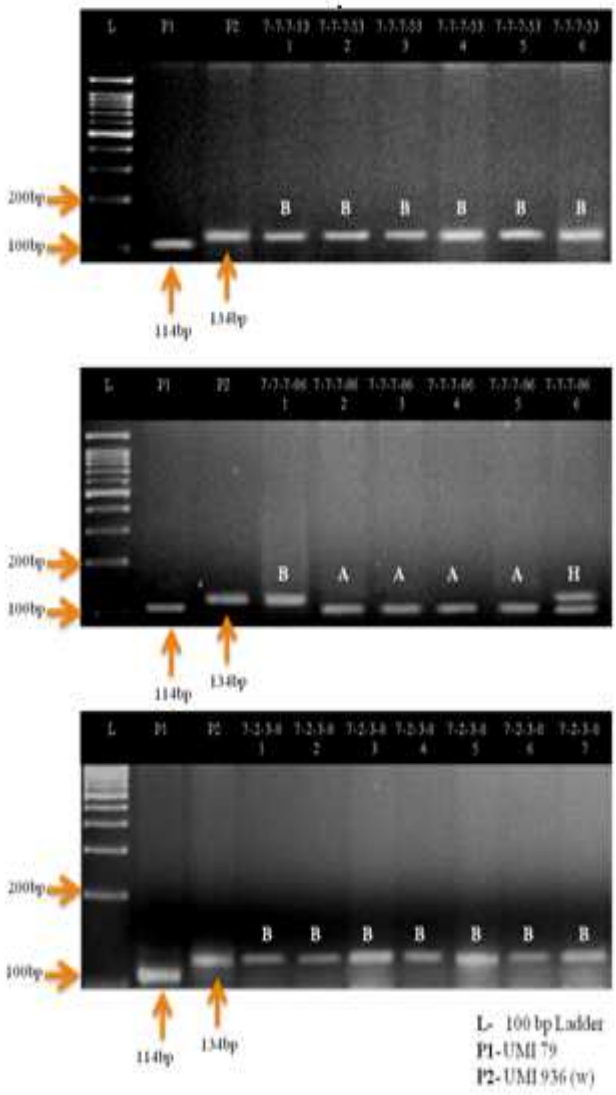

Figure 5: Segregation Pattern of SSR Matker nc013 in BC_3F_3 Progenies of (UMI79 x UMI936(w)) x UMI79

DISCUSSIONS

- Thirty eight phenotypically resistant individuals from 7-2-3 progeny and sixty five phenotypically resistant individuals from progeny no. 7-7-7 were selfed to generate the BC_3F_3 generation. From this population eight phenotypically superior progenies comprising sixty eight individuals were selected and genotyped with two SSR markers located on the chromosome 3 and chromosome 6.
- Among the 12 SSR markers reported to be located on different chromosomes (George *et al*., 2003; Nair *et al*., 2005; Sabry *et al*., 2006 and Kashmiri, 2010), two markers *viz.*, phi053 on chromosome 3 and nc013 on chromosome 6 which showed good polymorphism between the parental lines of the present study were used for the analysis of BC_3F_3 progenies.
- The genotyping of BC_3F_3 progenies for the marker phi053 (chromosome 3) revealed 6 progenies to be heterozygous (H), one progeny similar to UMI 79 and one progeny similar to UMI 936(w).

- For marker nc013 (chromosome 6), 5 BC_3F_3 progenies were found to be heterozygous (H) and 3 were similar to UMI 936(w).
- One BC_3F_3 progeny (7-7-7-53) was identified homozygous for UMI 936(w) allele for both the polymorphic SSR markers phi053 and nc013 and two progenies (7-7-7-85 and 7-2-3-8) were identified homozygous for UMI 936(w) allele for marker nc013 (chromosome 6) alone. These three lines can be designated as NILs (Near Isogenic Lines) resistant to the sorghum downy mildew disease and can be exploited in further maize hybrid breeding programmes.
- Four BC_3F_3 progenies (7-7-7-59, 7-7-7-84, 7-7-7-86 and 7-2-3-2) were identified heterozygous for the polymorphic markers phi053 and nc013. They are to be selfed and advanced into next generation to identify more NILs differing from each other for the resistance QTL alone.

Table 2: Genetic Constitution of BC_3F_3 Progenies

S. No.	BC3F3 Progeny	Number of Plants Screened	Number of Heterozygotes (H)		Number of Homozygotes Similar to UMI 79 (A)		Number of Homozygotes Similar to UMI 936(w) (B)	
			phi053	nco13	phi053	nc013	phi053	nc013
1	7-7-7-53	6	0	0	0	0	6	6
2	7-7-7-59	8	5	2	1	6	2	0
3	7-7-7-84	6	3	0	2	2	1	4
4	7-7-7-85	11	1	0	9	0	0	11
5	7-7-7-86	6	5	1	1	4	0	1
6	7-7-7-89	18	0	8	18	0	0	10
7	7-2-3-2	6	4	6	1	0	1	0
8	7-2-3-8	7	5	0	0	0	2	7

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